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PRINCIPAL INVESTIGATOR: Frederick P. Li, M.D.
Judy Garber, M.D.
Alexander Miron, Ph.D.

CONTRACTING ORGANIZATION: Dana-Farber Cancer Institute
Boston, MA 02115

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14. ABSTRACT Our purpose is to combine the well-established method of breast nipple fluid collection with new proteomics methodology for analyses of complex protein mixtures, in order to seek a better test for early breast cancer. The scope of our work is far-reaching, as our results could have a significant impact on the ability to detect occult breast cancers at earlier stages than is possible with current cancer detection methods. To date, we have completed a pilot test of 12 patients to optimize collection and lab storage and analysis conditions; collected 84 nipple aspirate fluid samples from controls; and collected 9 nipple aspirate fluid samples from cases. We have also modified the nipple aspirate fluid (NAF) collection protocol to collect NAF directly with capillary tubes. This new collection procedure is more efficient, providing more useable samples for lab analysis. We have run test samples with the SELDI-TOF methodology. Significant numbers of peaks were detected in those samples on IMAC40 chips thereby validating both the collection and analysis protocols. In summary, we have developed a workable protocol for nipple aspirate collection that produces consistent quantitative protein analysis. In the next year, we will continue sample collection and analyses.					
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INTRODUCTION:

When breast cancers are diagnosed by self-examination, clinical examination and/or mammography, the tumor is usually comprised of millions of genetically-unstable cancer cells, including subclones differing in responses to therapy. The goal of our project is to find a new method to detect occult breast cancers at earlier stages than is possible with current cancer detection methods. To achieve our goals, we have combined the well-established method of breast nipple fluid collection with the new proteomics methodology for analyses of complex protein mixtures, in order to seek a better test for early breast cancer. We aim to collect breast nipple fluids from 200 women with yet-untreated breast cancer (cases), and 200 cancer-free controls and perform proteomic analyses in 100 cases and 100 controls to identify nipple fluid protein patterns associated with the presence of breast cancer. Specific proteins and patterns will be validated by repeating the proteomic studies on a second blinded series of fluids from another series of 100 cases and 100 controls, link laboratory results with clinical and diagnostic data, and score the sensitivity and specificity of the assay. If successful, the proteomics assay might permit earlier breast cancer diagnosis, and thereby reduce disease morbidity and mortality.

BODY:

Task 1. Subject recruitment and consent (years 1 and 2)

- a. We modified our existing IRB-approved protocol (93-020: Collection and Laboratory Study of Breast Nipple Fluid Using a Nipple Suction Cup) to include proteomic analyses among the laboratory assays, renaming it 03-249. After receiving full IRB and DOD approval of 03-249, we have closed 93-020 for recruitment, leaving it open only for data analysis. There is no longer a need to collect under 93-020.
- b. We continue to obtain consent from eligible subjects and their physicians to collect breast nipple fluid samples and demographic and clinical data from 200 women with newly diagnosed breast cancer.
- c. In like manner, we continue to consent 200 cancer-free control subjects examined at the mammography centers within Dana-Farber/Harvard Cancer Center.

Task 2. Nipple fluid collection (years 1 and 2)

- a. We continue to use our previously developed methods to collect nipple fluids into capillary tubes from subjects. We have collected 93 NAF samples to date. Of these, 84 are samples from controls, and nine are from cases.
- b. Samples are being delivered to Dr Miron's laboratory where they are stored at -140 degrees C after recording the volume.

Task 3. Biomarker discovery in nipple fluid (years 1 and 2)

- a. We have optimized lab conditions to analyze NAF after collection. Optimization includes: (1) having established a NAF recovery protocol from the capillary tubes using a denaturing buffer and centrifugation, which is well suited for high-throughput studies; and (2) running test samples with the SELDI-TOF methodology. Significant numbers of peaks were detected in those samples on IMAC40 chips thereby validating both the collection and analysis protocols.
- b. Further analysis will be performed on a complete set of aspirates at the end of the collection phase of the study. We aim to perform proteomic analyses on 100 aliquots of nipple fluids from patients with breast cancer and 100 cancer-free women, using SELDI-TOF methodology using each of the chip surfaces in duplicate. *(No work has been done on this portion pending completion of the collection: such work can only be done in batches to minimize other sources of variation)*

Task 4. Identification and validation of nipple fluid biomarkers for cancer (years 2 and 3)

(To date, no work has been done on this task)

- a. Identify protein patterns in the arrays that differentiate nipple fluids obtained from cancerous breasts as compared with tumor-free breasts.
- b. Catalogue the protein markers present in nipple fluids from cancerous breasts that are absent from nipple fluids of cancer-free women.
- c. To validate the preliminary findings from the first set of samples, a third party will code the second series of 100 nipple fluid samples from breast cancer patients and 100 samples from cancer-free women.
- d. Repeat the SELDI-TOF analyses in a blinded manner on the 100 case samples and 100 controls.
- e. Analyze data for the 200 blinded samples and score the source of the sample as a cancer-bearing breast, normal breast or indeterminate origin.
- f. Break the sample codes and quantify the sensitivity and specificity of the assay in differentiating breast cancer cases from healthy controls.
- g. Identify causes of false-positive and false-negatives.

Task 5. Dissemination of results and, if appropriate, organize multi-center studies (year 3)

(To date, no work has been done on this task)

- a. Prepare manuscript(s) for peer-review and publication.
- b. If the proteomic analysis is shown to have high predictive value, develop a multi-center collaborative to re-confirm the results in a larger study.
- c. Seek additional funding to further refine the assay, and submit a Traditional Research Proposal.

KEY RESEARCH ACCOMPLISHMENTS:

- We have worked out a collection method that is efficient and obtains a high-yield of nipple aspirate fluid.
- We have determined that resuspending the aspirate in a small amount of PBS allows for full recovery of the sample.
- We have developed systematic protocol for collection that is easily adaptable to other sites.

REPORTABLE OUTCOMES:

None to date

CONCLUSIONS:

To date, we have developed a workable protocol for nipple aspirate collection that produces consistent quantitative protein analysis. We have completed a pilot collection phase to optimize collection and analysis methods. We have successfully collected 93 NAF samples. We are looking forward to continuing sample collection and beginning analysis.

REFERENCES:

Not applicable

APPENDICES:

Not applicable